Translation

PATENT COOPERATION TREATY

PCT/EP2003/009968

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 12186WO	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/EP2003/009968	International filing date (*day/month/year*) 08 September 2003 (08.09.2003)	Priority date (*day/month/year*) 11 September 2002 (11.09.2002)
International Patent Classification (IPC) or national classification and IPC G01N 33/68		
Applicant FRANKGEN BIOTECHNOLOGIE AG		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 13 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08 April 2004 (08.04.2004)	15 December 2004 (15.12.2004)
Name and mailing address of the IPEA/EP Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

Int[redacted]nal application No.
PCT/EP2003/009968

I. Basis of the report

1. With regard to the **elements** of the international application:*

☒ the international application as originally filed

☒ the description:

pages 1-107, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☒ the claims:

pages 1-20, as originally filed
pages _____, as amended (together with any statement under Article 19
pages _____, filed with the demand
pages _____, filed with the letter of _____

☒ the drawings:

pages 1/19-19/19, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☒ the sequence listing part of the description:

pages 78-107, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rule 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

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III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-20 (partly)

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _____ are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 1-20 (partly).

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

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IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:

See the supplemental sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-20 (partly)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Box III

The current claims 1 to 20 relate to an inordinately large number of possible methods and products, and owing to the inclusion of the very broad feature "or fragments thereof", only a small proportion of these are supported by the description in accordance with PCT Article 6 and/or can be regarded as having been disclosed in the application in accordance with PCT Article 5. In this instance the claims lack the proper support and the application lacks the requisite disclosure to such an extent that it did not seem possible to carry out a meaningful search covering the full range of subject matter for which protection is sought. The search was therefore directed to the parts of the claims that appear to be supported and disclosed in the above sense, that is those relating to the products themselves (i.e. the actual BHS-specific protein) and to its use in the claimed method. The examination in connection with PCT Article 33(2) and (3) was restricted to the subject matter that had been searched.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Box IV.3

The International Examining Authority concurs with the International Searching Authority in its objection on the grounds of lack of unity of invention (PCT Rule 13.1). The application covers the following groups of inventions which are not linked by a single general inventive concept:

1. Claims 1-20 (in part: ITM2A, i.e. SEQ ID Nos. 4 and 5)

Method for detecting the presence of a BHS-specific protein or fragment thereof in brain capillary endothelial cells, involving cDNA subtraction and differential hybridisation; method for detecting the presence of a BHS-specific protein or fragment thereof in brain capillary endothelial cells, involving isoelectric focussing; protein with BHS specificity or a fragment thereof having SEQ ID NO. 5; use of said protein in the preparation of a medicament for conveying substances through the blood-brain barrier; use of said protein in the preparation of an agent or medicament for diagnosing or treating diseases involving a dysfunction of the blood-brain barrier; agent for diagnosing diseases involving a dysfunction of the blood-brain barrier, containing said protein; agent for treating diseases involving a dysfunction of the blood-brain barrier, containing said protein; use of a DNA sequence SEQ ID NO. 4 in the preparation of an agent for diagnosing diseases associated with ischemic conditions.

2. Claims 1-20 (in part: S231, i.e. SEQ ID NOS. 6 and 14)

Method for detecting the presence of a BHS-specific protein or fragment thereof in brain capillary endothelial cells, involving cDNA subtraction and differential hybridisation; method for detecting the presence of a BHS-specific protein or fragment thereof in brain capillary endothelial cells,

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Box IV.3

involving isoelectric focussing; protein with BHS specificity or a fragment thereof having SEQ ID NO. 14; use of said protein in the preparation of a medicament for conveying substances through the blood-brain barrier; use of said protein in the preparation of an agent or medicament for diagnosing or treating diseases involving a dysfunction of the blood-brain barrier; agent for diagnosing diseases involving a dysfunction of the blood-brain barrier, containing said protein; agent for treating diseases involving a dysfunction of the blood-brain barrier, containing said protein; use of a DNA sequence SEQ ID NO. 6 in the preparation of an agent for diagnosing diseases associated with ischemic conditions.

3. Claims 1-20 (in part: FLJ13448, i.e. SEQ ID NOS. 15 and 19)

Method for detecting the presence of a BHS-specific protein or fragment thereof in brain capillary endothelial cells, involving cDNA subtraction and differential hybridisation; method for detecting the presence of a BHS-specific protein or fragment thereof in brain capillary endothelial cells, involving isoelectric focussing; protein with BHS specificity or a fragment thereof having SEQ ID NO. 19; use of said protein in the preparation of a medicament for conveying substances through the blood-brain barrier; use of said protein in the preparation of an agent or medicament for diagnosing or treating diseases involving a dysfunction of the blood-brain barrier; agent for diagnosing diseases involving a dysfunction of the blood-brain barrier, containing said protein; agent for treating diseases involving a dysfunction of the blood-brain barrier, containing said protein; use of a DNA sequence SEQ ID NO. 15 in the preparation of an agent for diagnosing diseases associated with ischemic conditions.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Box IV.3

4. Claims 1-20 (in part: NSE2, i.e. SEQ ID NOS. 22 and 23)

Method for detecting the presence of a BHS-specific protein or fragment thereof in brain capillary endothelial cells, involving cDNA subtraction and differential hybridisation; method for detecting the presence of a BHS-specific protein or fragment thereof in brain capillary endothelial cells, involving isoelectric focussing; protein with BHS specificity or a fragment thereof having SEQ ID NO. 23; use of said protein in the preparation of a medicament for conveying substances through the blood-brain barrier; use of said protein in the preparation of an agent or medicament for diagnosing or treating diseases involving a dysfunction of the blood-brain barrier; agent for diagnosing diseases involving a dysfunction of the blood-brain barrier, containing said protein; agent for treating diseases involving a dysfunction of the blood-brain barrier, containing said protein; use of a DNA sequence SEQ ID NO. 22 in the preparation of an agent for diagnosing diseases associated with ischemic conditions.

5. Claims 1-20 (in part: DRG-1, i.e. SEQ ID NOS. 26 and 27)

Method for detecting the presence of a BHS-specific protein or fragment thereof in brain capillary endothelial cells, involving cDNA subtraction and differential hybridisation; method for detecting the presence of a BHS-specific protein or fragment thereof in brain capillary endothelial cells, involving isoelectric focussing; protein with BHS specificity or a fragment thereof having SEQ ID NO. 27; use of said protein in the preparation of a medicament for conveying substances through the blood-brain barrier; use of said protein in the preparation of an agent or medicament

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Box IV.3

for diagnosing or treating diseases involving a dysfunction of the blood-brain barrier; agent for diagnosing diseases involving a dysfunction of the blood-brain barrier, containing said protein; agent for treating diseases involving a dysfunction of the blood-brain barrier, containing said protein; use of a DNA sequence SEQ ID NO. 26 in the preparation of an agent for diagnosing diseases associated with ischemic conditions.

6. Claims 1-20 (in part: TKA-1, i.e. SEQ ID NOS. 32 and 33)

Method for detecting the presence of a BHS-specific protein or fragment thereof in brain capillary endothelial cells, involving cDNA subtraction and differential hybridisation; method for detecting the presence of a BHS-specific protein or fragment thereof in brain capillary endothelial cells, involving isoelectric focussing; protein with BHS specificity or a fragment thereof having SEQ ID NO. 33; use of said protein in the preparation of a medicament for conveying substances through the blood-brain barrier; use of said protein in the preparation of an agent or medicament for diagnosing or treating diseases involving a dysfunction of the blood-brain barrier; agent for diagnosing diseases involving a dysfunction of the blood-brain barrier, containing said protein; agent for treating diseases involving a dysfunction of the blood-brain barrier, containing said protein; use of a DNA sequence SEQ ID NO. 32 in the preparation of an agent for diagnosing diseases associated with ischemic conditions.

7. Claims 1-20 (in part: PNOV-1, i.e. SEQ ID NOS. 49, 52 and 53)

Method for detecting the presence of a BHS-specific protein or fragment thereof in brain capillary endothelial cells,

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Box IV.3

involving cDNA subtraction and differential hybridisation; method for detecting the presence of a BHS-specific protein or fragment thereof in brain capillary endothelial cells, involving isoelectric focussing; protein with BHS specificity or a fragment thereof having SEQ ID NO. 53; use of said protein in the preparation of a medicament for conveying substances through the blood-brain barrier; use of said protein in the preparation of an agent or medicament for diagnosing or treating diseases involving a dysfunction of the blood-brain barrier; agent for diagnosing diseases involving a dysfunction of the blood-brain barrier, containing said protein; agent for treating diseases involving a dysfunction of the blood-brain barrier, containing said protein; use of a DNA sequence SEQ ID NO. 52 or 49 in the preparation of an agent for diagnosing diseases associated with ischemic conditions.

8. Claims 18-20 (in part: ARL-8, i.e. SEQ ID NOS. 35, 36 and 43)

Use of one or more DNA sequences selected from SEQ ID NOS. 35, 36 and 43 in the preparation of an agent for diagnosing diseases associated with ischemic conditions.

9. Claims 18-20 (in part: TSC-22, i.e. SEQ ID NOS. 54 and 55)

Use of one or more DNA sequences selected from SEQ ID NOS. 54 and 55 in the preparation of an agent for diagnosing diseases associated with ischemic conditions.

The reasons for this are as follows:

Inventions/groups 1 to 9 are not so linked as to form a single general inventive concept (PCT Rule 13.1). PCT Rule 13.1 in conjunction with PCT Rules 13.2 and 13.3 specifies that there must

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Box IV.3

be a general inventive concept linking the various (groups of) inventions. This linking concept should be a special technical feature which contributes to novelty and inventiveness in relation to the prior art.

The common inventive concept linking the above inventions or groups of inventions is a BHS-specific protein or fragment thereof. However, such proteins are well known in the prior art (see, for example, the abstract of document D1 (ITM2) and the description in the present application (page 2, first paragraph; page 51, third paragraph; page 55, last paragraph).

The applicant has not paid any additional fees. Claims or parts of claims relating to inventions in respect of which no international search report has been established cannot normally be the subject of an international preliminary examination (PCT Rule 66.1(e)). In its capacity as International Preliminary Examining Authority the EPO does not carry out a preliminary examination for subject matter that has not been searched. Therefore in the present instance only the first invention will be examined.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
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V. **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-6, 8-11, 13-20	YES
	Claims	7, 12	NO
Inventive step (IS)	Claims	1-6, 9-11, 14-17	YES
	Claims	7-8, 12-13, 18-20	NO
Industrial applicability (IA)	Claims	1-20	YES
	Claims		NO

2. Citations and explanations

Reference is made to the following documents:

D1: Journal of Biological Chemistry (09-08-1996), 271(32), 19475-19482

D2: Molecules and Cells (31-12-2001), 12(3), 391-397

D3: WO-A-0168848

The intermediate document "Brain Research, 2003, Vol. 967, No. 1-2, pp. 11-18" (document D4) was published after the priority date of the present application and before its filing date, and is therefore only relevant if the priority claim proves to be invalid. The priority claim has not yet been checked.

1. PCT Article 33(2) and (3)

1.1 The subject matter of **independent claim 1 is considered to be novel (PCT Article 33(2))** over the prior art according to document D1 because the claimed method for detecting Itm2A in brain capillary endothelial cells is used.

D1 discloses a method for isolating new markers of chondro-osteogenic differentiation, such as the marker Itm2, involving the isolation of condyles followed by cDNA subtraction in organ cultures and prenatal mouse condyles, and also differential hybridisation, expression verification

in various clones (page 19478, column 1, last paragraph), followed by a comparison in order to identify new markers (see the abstract).

Thus the technical problem to be solved in the light of D1 can be seen as that of providing an alternative use for the known method.

This problem is solved by a method as described above.

Document D2 describes the expression if Itm2A in muscle cells, but not in brain cells (page 396, column 1, second paragraph, and figure 4).

Document D3 shows a sequence 184 (figure 184, claim 11) and a sequence which is 80% homologous to sequence 184, and the use thereof in the diagnosis of diseases such as tumours. Sequence 184 is in turn 91.6% homologous to SEQ ID NO. 5 in the present application.

Hence it is not obvious from the prior art teaching that Itm2A is a protein which is expressed in brain capillary endothelial cells. **Independent claim 1 is therefore considered inventive** (PCT Article 33(3)).

The same applies to **dependent claims 2 to 6.**

1.2 **Independent claim 7** relates to the BHS-specific protein Itm2A and **is not considered novel** (PCT Article 33(2)). As indicated in the application itself (page 2, first paragraph; page 51, third paragraph; page 55, fourth paragraph), BHS-specific proteins are well known in the prior art.

For example, Itm2, which is described in the present application (page 55, last paragraph) as a BHS protein, is disclosed in document D1 (see the abstract).

Claimed products must be novel *per se*. The simple fact of

being produced by a novel process does not make them novel.

The subject matter of **dependent claim 8 is novel** because none of the prior art documents disclose a protein that has SEQ ID NO. 5.

Document D1 discloses a protein (see figure 3) which is 91.6% homologous to SEQ ID NO. 5.

Document D3 shows a protein sequence (see figure 184 and claim 11) which is 91.6% homologous to SEQ ID NO. 5.

Since it is not clear what surprising technical effect results from the minor homology difference between the sequences, the subject matter of **dependent claim 8 is not considered inventive (PCT Article 33(3))**.

1.3 The subject matter of **independent claim 9 is considered novel** because none of the prior art documents disclose a method for detecting the presence of Itm2A in brain capillary endothelial cells that involves isoelectric focussing and mass spectrometry analysis.

Document D2 describes the expression if Itm2A in muscle cells, but not in brain cells (page 396, column 1, second paragraph, and figure 4).

It is not obvious from the prior art teaching that Itm2A can be detected in brain capillary endothelial cells. The subject matter of **independent claim 9**, like that of independent claim 1, is therefore considered **inventive**.

The same applies to **dependent claims 10 and 11**.

1.4 The subject matter of **independent claim 12 is not considered novel** for reasons similar to those applying to independent claim 7.

Dependent claim 13, like dependent claim 8, **is not considered inventive.**

1.5 The subject matter of **independent claim 14 is considered novel and inventive** because none of the prior art documents disclose the expression of Itm2A as a BHS-specific protein. Document D2 even tends to suggest the opposite by stating that no Itm2A expression was found in the brain (see also point 1.1 above).

1.6 The subject matter of **independent claim 15** is considered **novel and inventive** for reasons similar to those applying to independent claim 14.

1.7 The subject matter of **independent claim 16** is considered **novel and inventive** for reasons similar to those applying to independent claim 14.

1.8 The subject matter of **independent claim 17** is considered **novel and inventive** for reasons similar to those applying to independent claim 14.

1.9 The subject matter of **independent claim 18 is considered novel** because none of the prior art documents disclose the use of the DNA sequence with SEQ ID NO. 4 in the preparation of an agent for diagnosing diseases associated with ischemic conditions.

Document D3 shows a sequence 183 (see figure 183 and claims 2 and 3) which is 80.43% homologous to SEQ ID NO. 4 in the present application. Claims 2 and 3 of D3 claim a sequence that has at least 80% nucleic acid correspondence with sequence 184. The disclosed sequences are used to diagnose diseases (page 82, line 29 ff.) such as tumours of various types (example 16).

The subject matter of **independent claim 18 is not considered inventive** because it is not clear what

surprising technical effect is associated with the claimed subject matter in relation to D3.

The same applies to **dependent claims 19 and 20**, the additional technical features of which are already known from document D3 (page 82, line 29 ff., and example 16).